AFOSR-TR-97-0701

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		FINAL 15 Feb 96 to 14 Feb 98

4. TITLE AND SUBTITLE

IMMUNOTOXICOLOGY OF EXPOSURE TO JP-8 JET FUEL

5. FUNDING NUMBERS

F49620-96-1-0075
2312/AS
61102F

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BOLLING AFB DC 20332-8050

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10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited.

19971217 034

13. ABSTRACT *(Maximum 200 words)*

Chronic jet fuel exposure could be detrimental to Air Froce personnel, by not only adversely affecting their work performance but also by predisposing these individuals to increased incidences of infectious disease, cancer and autoimmune dysfunctions. Chronic exposure to jet fuel has been shown to adversely affect human liver function, to cause emotional dysfunction, to cause abnormal electroencephalograms, to cause shortened attention spans, and to decrease sensorimotor speed. Currently, there are no standards for personnel exposure to jet fuels of any kind, let along JP-8 jet fuel. Kerosene based petroleum distillates have been associated with hepatic, renal, neurologic and pulmonary toxicity in animals models and human occupational exposures. The U.S. Department of Labor, Bureau of Labor statistics estimates that over 1.3 million workers were exposed to jet fuels in 1992. Thus, jet fuel exposure may not only have serious consequences for USAF personnel, but also may have potential harmful effects upon a significant number of civilian workers. Short-term (7 day) JP-8 jet fuel exposure causes lung injury as evidenced by increased pulmonary resistance, a decrease in bronchoalveolar lavage concentrations of substance P, increased wet lung/body weight ratio, and increased alveolar permeability. Long-term exposures,

14. SUBJECT TERMS	15. NUMBER OF PAGES
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
U	U	U	UL

NSN 7540-01-280-5500

DTIC QUALITY INSPECTED 3

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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F49620-96-1-0075, Dr Harris, Univ of Arizona

although demonstrating evidence of lung recovery, results in injury to secondary organis such as liver, kidneys and spleen.

FINAL TECHNICAL REPORT

"IMMUNOTOXICOLOGY OF EXPOSURE TO JP-8 JET FUEL"

#F49620-96-1-0075

December 1997

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ABSTRACT

Chronic jet fuel exposure could be detrimental to Air Force personnel, by not only adversely affecting their work performance but also by predisposing these individuals to increased incidences of infectious disease, cancer and autoimmune dysfunctions. Chronic exposure to jet fuel has been shown to adversely affect human liver function, to cause emotional dysfunction, to cause abnormal electroencephalograms, to cause shortened attention spans, and to decrease sensorimotor speed. Currently, there are no standards for personnel exposure to jet fuels of any kind, let alone JP-8 jet fuel. Kerosene based petroleum distillates have been associated with hepatic, renal, neurologic and pulmonary toxicity in animals models and human occupational exposures. The U.S. Department of Labor, Bureau of Labor Statistics estimates that over 1.3 million workers were exposed to jet fuels in 1992. Thus, jet fuel exposure may not only have serious consequences for USAF personnel, but also may have potential harmful effects upon a significant number of civilian workers. Short-term (7 day) JP-8 jet fuel exposure causes lung injury as evidenced by increased pulmonary resistance, a decrease in bronchoalveolar lavage concentrations of substance P, increased wet lung/body weight ratio, and increased alveolar permeability. Long-term exposures, although demonstrating evidence of lung recovery, results in injury to secondary organs such as liver, kidneys and spleen.

We have observed that short-term (7 days) exposure of C57BL6 mice to low concentrations (100-500 mg/m^3) of JP-8 jet fuel results in profound and significant alterations on the immune system. Organ weights (spleen and thymus) and total cell numbers recovered from each of the major immune system organs (spleen, thymus, lymph nodes, bone marrow and peripheral blood) were significantly reduced. Flow cytometric analyses revealed that T cell populations were lost with significant increases in inflammatory and B cell populations in these organs. JP-8 exposure resulted in a significant depression of immune function (as measured by proliferative assays) of the residual cells which could not be overcome by the addition of exogenous immune response modifiers, and which may be indicative of a generalized inflammatory response. Further, JP-8 exposure resulted in a loss of natural killer (NK) cell function, lymphokine activated killer (LAK) cell activity, and cytotoxic T lymphocyte (CTL) capacity. Additional analyses demonstrated that precursor cells for both cytotoxic and helper T cells had been affected by the exposures.

Mice exposed to a 7 day JP-8 jet fuel exposure exhibited an ability to recover from the immune alterations observed. Although immune organ weights recovered to normal levels within a week of cessation of exposure, up to one month was required to return immune organ cell numbers to baseline levels in the spleen and thymus. Cell numbers in the bone marrow, lymph nodes and peripheral blood recovered more quickly. Significantly, recovery of immune function was more profoundly affected, taking more than one month to recover to normal levels. Thus, short-term, low concentration exposures to JP-8 jet fuel resulted in long lasting immune alterations.

Interestingly, treatment of the mice with the neuropeptide substance P (1 uM, 15 minutes) immediately after JP-8 exposure was able to reverse many of the observed effects. That is, substance P administration was able to protect/reverse the effects of JP-8 jet fuel exposure on immune organ weights, immune organ numbers, and immune function. Confirmation of the role of substance P in this process was found in that administration of substance P inhibitors generally made the effects of JP-8 exposure even worse.

Tow recent studies have also provided some interesting preliminary data. In the first study, expsoure of mice to JP-8+100 jet fuel (1000 mg/m^3) also induced immunotoxicological damage comparable to that observed with JP-8 jet fuel alone. In the second study, injection of mice with substance P 15 minutes prior to JP-8 exposure was as effective in preventing the immunotoxicological effects of JP-8 jet fuel as administering substance P after the JP-8 exposure period. Studies are currently underway to identify the active components/metabolites of substance P responsible for the protective effects.

Thus, exposure of individuals to JP-8 jet fuel may result in increased risk of infectious disease, cancer and autoimmune disease. However, it may be possible to reverse or prevent many of these effects through the administration of a common neuropeptide. It is absolutely critical to ascertain and understand the potential consequences of immune function alterations as it pertains to the short-term and long-term health and well-being of exposed personnel. The results obtained in these studies should have significant implications for the health, well-being and medical treatment of JP-8 exposed individuals.

OBJECTIVES/STATEMENT OF WORK:

The Specific Aims of the grant for Year 02 were as follows (including the completion of portions of Specific Aims #1 and #2).

Specific Aim #3. : Effect of Substance P (SP) Administration on Protection From and Reversibility of Effects of JP-8 Exposure (Year 01-02).

B.SP administration prior to JP-8 exposure
- a. delayed versus immediate administration
- b. SP concentration response

During year 02 of the grant we have finished all work on Specific Aims #1 and #2. Due to a change in priorities after our annual AFOSR JP-8 jet fuel conference the work on this specific aim has been somewhat delayed, although not eliminated. Part (A) of the specific aim has been completed and part (B) has been initiated and should be completed by January (two experiments have been completed and the third repetition has been planned).

Specific Aim #4. : The Effect of JP-8 Exposure on the Development and/or Progession of Infectious Disease, Cancer, and Autoimmunity Utilizing Animal Models (Year 02).

A. Effects in a cancer system model
- a.B16 melanoma/metastasis model
  - i. on tumor development
  - ii. on tumor progression

B. Effects in an infectious disease system model
- a. influenza model

C. Effects in an autoimmunity system model
- a. MRL-lpr/lpr and NZB models

Due to a change in priorities after our annual AFOSR JP-8 jet fuel conference this specific aim was eliminated, in order to continue studies on substance P and the effects of other sources of jet fuel.

STATUS OF EFFORT:

We have continued our investigation of the immunotoxicological effects of exposure to JP-8 jet fuel, making use of murine animal models. Our experiments have revealed that the immune system may be the most sensitive indicator (and predictor) of toxicological effects in the exposed individual. Short-term, low-concentration JP-8 exposure resulted in a profound and significant alteration in many immune parameters measured (immune organ weights, immune cell numbers, mitogenic responses, CTL, NK and LAK effector cell mechanisms, and helper and cytotoxic T cell precursors). We also have continued to explore the mechanism of the protective effects of substance P administration on JP-8-induced immunotoxicity.

The work from this grant has resulted in several manuscripts being published, as well as the filing of a patent application (please see below). Our collaboration with Dr. Mark Witten (also of the University of Arizona and also funded by the AFOSR) continues to operate smoothly, maximizing experimental outcomes while minimizing animal use.

The results from this grant has aided us in understanding the actions of substance P on protection of the immune system from the immunotoxicological effects of JP-8 jet fuel exposure, as well as providing us with an insight into the potential effects of JP-8 on clinical disease manifestations.

ACCOMPLISHMENTS/NEW FINDINGS:

During the past three years of experimentation we have examined the immunotoxicological effects of JP-8 jet fuel exposure. Inbred C57BL6 mice were exposed to varying concentrations (either 500, 1000 or 2500 mg/m^3) of aerosolized JP-8 jet fuel for a period of 7 days with an exposure period of 1 hour per day. Animal exposure was performed via nose-only presentation while the animals were held in individual subject loading tubes. The tubes were nose cone fitted to receiving adapters that originated from a common anodized aluminum exposure chamber. Nose only exposure was utilized to minimize ingestion of jet fuel during self grooming. Animals were rotated on a daily basis through the 12 adapter positions on the exposure chamber. This rotation was done to minimize proximity to the jet fuel source as a variable in exposure concentration or composition. Exposure concentration was determined by a seven stage cascade impactor, and was measured after each exposure (1,2). 24 hours after the last exposure the animals were sacrificed and examined for changes in immune system composition and function. The major immune system organ systems (i.e., spleen, thymus, lymph nodes, blood and bone marrow) were recovered and examined for changes in organ weight, total cell numbers, immune cell components (by differential histochemical staining), and lymphocyte subpopulations by flow cytometric analyses. Assays were also performed to assess any changes in immune function in these organs. In some experiments the animals were administered an aerosolized concentration of the neuropeptide substance P (SP, 1 uM) for 15 minutes immediately following the JP-8 exposure in an effort to protect from or reverse the effects of JP-8 exposure. As controls, other animals were exposed only to air plus/minus SP (i.e., no JP-8 exposure). These

studies have resulted in one published manuscript, two other manuscripts accepted and in press, two manuscripts in preparation, and three abstracts (please see attachments).

The following significant observations were obtained during the two years of work on this grant project. The findings from these studies are summarized as follows (please see attached manuscripts for complete details).

Manuscript #1 (Harris et al, Immunotoxicological Effects of JP-8 Jet Fuel Exposure, Toxicology & Industrial Health 13(1):43, 1997).

1. JP-8 exposure results in significant depression in immune organ wet weights (spleen and thymus).

2. JP-8 exposure results in significant losses of immune organ cell numbers (spleen, thymus, lymph nodes, peripheral blood and bone marrow).

3. JP-8 exposure causes a significant loss of immune function, as assessed by mitogenic responses, which cannot be overcome by exogenous growth factors.

4. JP-8 exposure has significant effects on the immune system at concentration exposures as low as 100 mg/m^3 (thymus only).

5. The effects of JP-8 on the immune system are concentration-dependent. The majority of the effects of JP-8 exposure on the immune system are observed to begin at concentration exposures between 250-500 mg/m^3. Concentration exposures of 2500 mg/m^3 are generally thought to be directly toxic to the immune system.

6. No significant differences were observed in immune system effects based on gender of the exposed mice (i.e., male and female animals demonstrated comparable effects).

7. No significant differences were observed in immune system effects using either normal C57Bl/6 mice or enzyme-deficient congenic mice (deficient in two enzymes thought to be involved in hydrocarbon metabolism), indicating that the effects on the immune system were so severe that loss of these putatively important protective enzyme pathways was not relevant.

Manuscript #2 (Harris et al, Short-Term Exposure to JP-8 Jet Fuel Results in Longterm Immunotoxicity, Toxicology & Industrial Health 13(4), 1997).

8. Short-term exposures to JP-8 jet fuel results in depressions in wet weights of spleen and thymus which recover within 1 week, although some overcompensation occurs in the thymus.

9. Short-term exposure to JP-8 jet fuel results in significant losses of immune cells from the spleen which takes up to 1 month to recover to normal. Cell losses from the thymus however, recover more quickly.

10. Short-term exposure to JP-8 jet fuel does not appear to have any significant long-term effects on immune cell numbers isolated from lymph nodes, peripheral blood or bone marrow.

11. Short-term exposure to JP-8 jet fuel has significant and long-lasting effects on immune function. The higher the exposure concentration, the longer it takes for the immune system to recover (4 weeks or longer).

12. Immune system effects due to short-term (7 day) exposures are reversible (although not completely) after 3-4 weeks, indicating that even short-term, low concentration exposures have long-lasting effects on the immune system.

Manuscript #3 (Harris et al, Protection from JP-8 Jet Fuel Induced Immunotoxicity by Adminstration of Aerosolized Substance P, Toxicology & Industrial Health 13(4), 1997).

13. The effects of JP-8 exposure on the immune system can be reversed/prevented by administration of substance P (1 uM, 15 minutes) immediately after the jet fuel exposure.

14. Concentrations of substance P as low as 1 nM have significant protective effects against JP-8 induced immune system effects.

15. The effects of JP-8 exposure on the immune system are made worse by administration of substance P inhibitors.

Manuscript in preparation

16. JP-8 exposure results in the complete loss of natural killer (NK) cell function, which is long-lasting and results in the inability to give rise to lymphokine-activated killer (LAK) cell activity.

17. JP-8 exposure results in the complete loss of cytotoxic T lymphocyte (CTL) function.

18. JP-8 exposure results in the significant suppression of the ability of precursor T cells to give rise to lymphokine producing T cells (i.e., helper T cells).

19. JP-8 exposure results in the significant suppression of the ability of precursor T cells to give rise to cytotoxic T cells (i.e., CTL).

Manuscript in preparation

20. Initial evidence from long-term exposures (28 days) indicates that the immune system has some ability to compensate for the damage inflicted upon it by JP-8, although not in its entirety.

Manuscript in preparation

21. Preliminary experiments have indicated that exposure of mice to JP-8+100 jet fuel is just as detrimental as exposure to standard JP-8 jet fuel in terms of its effects on the immune system. Experiments are also currently underway to examine the effects of exposure to a synthetic JP-8 jet fuel blend (hydrocarbons only).

Manuscript in preparation

22. Preliminary results have demonstrated that injection of mice with substance P 15 minutes prior to JP-8 jet fuel exposure is just as effective at protecting the immune system from the detrimental effects of JP-8 jet fuel exposure as administration post-exposure. Experiments are underway to determine the length of time prior to JP-8 exposure that substance P can be given, and to indentify the active components/metablites of substance P responsible for its protective actions.

From the experiments that have been performed to date it appears that the immune system is a sensitive indicator of toxicological damage (both to the immune system as well as to other physiological systems) incurred by the individual due to JP-8 jet fuel exposure. The results summarized above (and the reprints/preprints that are included with this report) have indicated that exposure to JP-8 jet fuel, even at low concentrations and even for short periods of time, has significant and profound effects on the immune system. JP-8 should be considered a significant immunotoxicant. It would be expected that such changes in immunocompetence that have been observed after JP-8 exposure would have significant effects on the exposed individual's health and may adversely affect his/her susceptibility to infectious disease, as well as possibly the development and/or progression of cancer and autoimmune disease.

PERSONNEL SUPPORTED:

David T. Harris, Ph.D.	Principle Investigator
Thomas Tsang, Ph.D.	Postdoctoral Fellow
Farha Vasanwala, BS	Doctoral Graduate Student
Debbie Sakiestewa, BS	Senior Research Technician

PUBLICATIONS:

The following publications either are in the process of being submitted or have been accepted for publication and are in press or have been published.

1. Harris et al, Immunotoxicological Effects of JP-8 Jet Fuel Exposure, Toxicology & Industrial Health 13(1):43, 1997.

2. Harris et al, Short-Term Exposure to JP-8 Jet Fuel Results in Longterm Immunotoxicity, in press, Toxicology & Industrial Health 13(4), 1997.

3. Harris et al, Protection from JP-8 Jet Fuel Induced Immunotoxicity by Adminstration of Aerosolized Substance P, in press, Toxicology & Industrial Health 13(4), 1997.

4. Harris, DT, D. Sakiestewa, R. Robledo and M. Witten. JP-8 jet fuel exposure results in loss of cell-mediated immunity in mice. (Submitted for publication)

5. M.L. Witten, *D.T. Harris*, R.F. Robledo and D. Srinivasan. Aerosolized [SAR9, MET(O2)11]-Substance P Causes Immunostimulation in Three Different Animal Models. International Substance P Conference, Cairns, Australia, September 1997.

INTERACTIONS/TRANSITIONS:

1. Meetings, Conferences, Seminars

AFOSR Toxicology Conference in Dayton, OH, December 1997
AFOSR JP-8 Meeting at The University of Arizona, Tucson, AZ; May 1997

2. Consultants (for whom I consult)

Cord Blood Registry, Inc.
Tel-Tech, Inc.
Medical Advisory Board, Canadian J. Clin. Med-Medical Scope Monthly, 1997-98
Member, Advisory Committee, Dr.. J. Enriquez NIH grant on "Mucosal Immunity to C. parvum", Univ. Arizona, 1995-2000
Ad-Hoc reviewer for Toxicology & Industrial Health

3. Transitions

None to date. However, the Air Force has shown considerable interest (i.e., Wright Patterson Labs) with regard to the potential effects of JP-8 exposure on human personnel, as well as the actions of substance P in protecting from any immunotoxicological effects. The joint US/Norway cooperative has also expressed considerable interest in these findings and have received copies of all data and manuscripts.

PATENTS/INVENTIONS:

A patent application entitled "Substance P for Treatment of Immunosuppression" was filed with the United States Patent Office in July 1996. In July 1997 the patent was amended to be a worldwide patent. The patent originated from work performed on our Air Force Office of Scientific Research sponsored grant entitled, "Immunotoxicology of Exposure to JP-8 Jet Fuel". The patent, as well as our grant work, has been performed in conjunction with Dr. Mark Witten (Dept. of Pediatrics, University of Arizona). Because of the laws involving public disclosure during the patent process, we have been slightly delayed in some of our publications related to substance P. However, as we are now in discussions with several pharmaceutical companies regarding this discovery, this issue should be shortly resolved. This discovery may have significant clinical benefits in terms of boosting the immune systems of individuals that are immunocompromised (e.g., AIDS patients, cancer patients, aged individuals, etc.), as well as been of benefit to those exposed to environmental toxicants (e.g., hydrocarbons, cigarette smoke, etc.). Further, we are now in discussions with another pharmaceutical company with regard to licensing for effects on cognitive functions.

HONORS/AWARDS:

1997	Elected to "Who's Who in America"
1997	Elected to "Who's Who in Medicine and Healthcare"
1997	Granted, 1 year Sabbatical
1997	Elected to "Who's Who in the West"
1997	Elected to "American Men and Women in Science"
1998	Elected to "Who's Who in the West"
1998	Elected to "Who's Who in America"
1998	Elected to "Who's Who in the World"
1998	Elected to "Who's Who in Science and Engineering"
1997	Advisory Board, Canadian Journal of Clinical Medicine